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Research Article

Phytochemical Screening and In-vivo Antipyretic Activity of the Methanol Leaf-Extract of *Bombax Malabaricum* DC (Bombacaceae)

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Abstract

Purpose: To investigate the antipyretic activity of the methanol extract of *Bombax malabaricum* leaves (MEBM) in rats.

Methods: Baker's yeast was used to induce fever in Wistar rats which were divided into four groups. The animal groups were thereafter administered MEBM (200 mg/kg), MEBM (400 mg/kg), paracetamol (reference standard, 150 mg/kg) and 1 % Tween 80 (control), respectively. The body temperature of the rats was measured rectally over a period of 8 h. MEBM was also phytochemically screened for alkaloids, steroids, carbohydrates, tannins, fixed oils, proteins, triterpenoids, deoxy-sugar, flavonoid, cyanogenetic and coumarin glycosides.

Results: MEBM (200 mg/kg and 400 mg/kg) significantly reduced yeast-induced pyrexia ($p < 0.05$, $p < 0.01$, respectively). Phytochemical tests showed the presence of steroids, carbohydrates, tannins, triterpenoids, deoxy-sugars, flavonoids and coumarin glycosides.

Conclusion: The methanol extract of *Bombax malabaricum* leaves possesses significant antipyretic activity.

Keywords: Antipyretic activity, Baker's yeast, *Bombax malabaricum*, Phytochemical screening

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INTRODUCTION

Fever is a common medical symptom associated primarily with elevation of body temperature and is often accompanied by certain sickness-related behavioural features such as depression, sleepiness, lethargy, hyperalgesia, anorexia, etc. A number of disease conditions such as infections, skin inflammation, immunological disorders, tissue destruction, cancer, metabolic disorders, and reaction to incompatible blood products, usually accompany fever [1]. Mechanistically, the hypothalamus of the brain controls the heat effector mechanism via the autonomic nervous system, either by increase of heat production (through increased muscle tone or shivering) or prevention of heat loss by vasoconstriction [1]. There is controversy regarding the usefulness of fever; however, high temperature is always considered a medical emergency due to its serious side effects such as intracranial haemorrhage, sepsis, Kawasaki syndrome, thyroid storm, and serotonin syndrome [1].

The search for an ideal antipyretic drug is a never-ending challenge. Paracetamol and other popular synthetic antipyretic drugs have several side effects [2]. Therefore, it is worth searching for herbal materials that are equally efficacious but less toxic and comparatively free from side effects, as substitutes for synthetic drugs such as paracetamol.

The plant, *Bombax malabaricum* DC. (silk cotton tree) {Synonym: *Bombax ceiba* L.; *Salmalia malabaricum* Schott & Endl.}, belongs to the family Bombacaceae. It has been shown to be important in traditional medicine [3]. It is a lofty deciduous tree of up to 40 meters in height; its young stems and branches are covered with stout, hard prickles; its leaves are large, spreading, glabrous and digitate; leaflets are 5-7, lanceolate and 10-20 cm long; its flowers numerous, large, fleshy, bright crimson, yellow or orange, clustered at the end of the branches while its capsules are oblong-ovoid,

containing many seeds with long, dense silky hairs. It is found in the tropical and subtropical regions of the world. The plant is economically important as a source of cotton and timber, including its use for the production of match-sticks. [3].

The leaves are used traditionally in inflammation and cutaneous trouble [3,4]. Its leaf extract exhibits significant antifungal activities against ringworm infection while the bark gum contains catechutannic acid [5]. Dar *et al* [6] isolated antioxidant and analgesic mangiferin from the leaves of the plant. The root contains bombamalones A-D, bombamaloside, isohemigossypol-1-methyl ester, 5,2-*O*-methylisohemigossylic acid lactone, 6-bombaxquinone B and lacinilene C [7] while the petal contains orange-red anthocyanin pelargonidin-5- β -D-glucopyranoside and cyanidine-7-methyl ether-3 β -glucopyranoside. The root-bark contains lupeol, β -sitosterol, 8-formyl-7-hydroxy-5-isopropyl-2-methoxy-3-methyl-1,4-naphthaquinone, isohemiglogossyol-1-methyl ether and 7-hydroxycadalene [3]. Lupeol, β -sitosterol and β -sitosterol-D-glucoside have been isolated from its stem bark while lupeol, β -sitosterol and two naphthaquinones were obtained from the root bark of the plant [8].

To the best of our knowledge, there is no report on the antipyretic activity of the methanol extract of the leaves of *Bombax malabaricum*. The purpose of the present work, therefore, was to investigate the methanol extract of *Bombax malabaricum* leaves (MEBM) for its antipyretic activity. Phytochemical screening of the extract was also carried out.

EXPERIMENTAL

Chemicals and reagents

Methanol was obtained from CDH, India. Paracetamol was obtained from GlaxoSmithKline, India. Other chemicals and reagents used were of analytical grade.

Plant material

The fresh leaves of *Bombax malabaricum* were collected from the city of Azamgarh, Uttar Pradesh, India during the month of May 2008 and were identified by Dr HJ Chowdhery, a taxonomist and Joint Director, Central National Herbarium (CNH), Botanical Survey of India, Howrah, India. The voucher specimen (no. CNH/1-I(314)/2009-Tech II/356/346) was deposited in the herbarium of CNH, Botanical Survey of India, Howrah, India for future reference. The leaves were dried under the shade, powdered and then stored in an air-tight container prior to use.

Preparation of extract

The powdered leaves were extracted in a Soxhlet apparatus using methanol as solvent. The solvent was removed from the extract in a rotary vacuum evaporator and the extract subsequently dried in a vacuum oven at 45 °C to obtain a solid mass of the crude extract which was kept in a desiccator prior to use in the animal studies.

Preliminary phytochemical screening

Preliminary phytochemical screening for alkaloids, steroids, carbohydrates, tannins, fixed oils, proteins, triterpenoids, deoxy-sugar, flavonoid, cyanogenetic and coumarin glycosides carried out on the extract according to the procedures of Khandelwal [9]. Test for alkaloids carried out using Mayer's, Dragendorff's, Wagner's and Hager's reagents. Presence of steroid was confirmed with Libermann-Burchard, Salkowski, and Libermann's reactions. Test for carbohydrate was undertaken using Fehling's, Benedict's, Molish's, Tollen's and iodine tests. Keller-Killiani test was done to confirm the presence of deoxy-sugar. Noller's reagent was used to test for triterpenoids. Shinoda and Wolform tests were used for the identification of flavonoids. The presence of tannins was determined by reaction with 5% ferric chloride, acetic acid, lead acetate, gelatin, dilute potassium permanganate,

potassium dichromate solution, bromine water and dilute nitric acid. For cyanogenetic glycoside, Guignard reaction was used whereby a filter paper strip was soaked in 10 % picric acid and 10 % sodium carbonate, respectively. It was kept in the slit of a cork over the moistened extract in a conical flask. Brick red or maroon colour confirms the positive test for cyanogenetic glycosides. Blue or green fluorescence indicates a positive test for coumarin glycosides when the alcoholic extract is rendered alkaline. The extract was tested for protein using biuret and ninhydrin tests and also by reacting with Million's reagent. The presence of fixed oils by means of spot and saponification tests.

Test animals

Wister rats of either sex and weighing 120 - 130 g were used for the antipyretic test. The animals were kept under controlled conditions (temperature, 25 ± 2 °C; light/dark cycle, 12/12 h) and fed with standard pellet diet and water *ad libitum*. The study received prior approval from the Institutional Animal Ethical Committee, Pharmacy College, Azamgarh, India (approval reference no. 937/c/06/CPCSEA). Animal handling was followed the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) [10].

Preparation of extract for animal studies

The crude extract (MEBM, 600 mg) was suspended in 1 % aqueous Tween 80 (15 ml). Control treatment was with 1 % Tween 80 while the reference standard was paracetamol (250 mg) suspended in 1% Tween 80 (17 ml).

Induction of pyrexia

Antipyretic activity was measured by slightly modifying the method described by Dewan *et al* [11]. The rats were divided into four groups of six animals each. Rectal temperature was measured by introducing a 3 cm digital thermometer (Model MT-101, N & B Medical,

Delhi) coated with glycerin (lubricant) into the rectum. Pyrexia (10 ml/kg) was induced by intra-subcutaneous injection of 20 % Baker's yeast suspended in 0.9 % saline.

Drug administration

Four hours after yeast injection, the animal groups received orally MEBM (400 mg/kg or 200 mg/kg), paracetamol (reference standard (150 mg/kg) and 1 % Tween 80 (control), respectively. Body temperature was measured via the rectum hourly from 0 to 8 h [11].

Statistical analysis

All the results are expressed as the mean \pm S.E.M. The data were analyzed for statistical significance by one-way analysis of variance (ANOVA) followed by Tukey test using computerized GraphPad Prism, version 4.03 software (Graph Pad Software Inc). Values of $p < 0.05$ were considered statistically significant.

RESULTS

Phytochemical screening

The outcome of the preliminary phytochemical screening of the extract showed the presence of steroids, carbohydrates, tannins, triterpenoids, deoxy-sugar, flavonoids and coumarin glycosides.

Antipyretic activity

Administration of Baker's yeast produced an increase in the body temperature of the rats from normal (37.66 ± 0.02 °C) to 38.69 ± 0.03 °C within four hours of yeast injection. Fig 1 shows that maximum temperature was attained in the control group at 1 h (i.e., 5 h after yeast administration) with a value of 39.2 ± 0.04 °C. MEBM (200 mg/kg, 400 mg/kg) and paracetamol reduced the body temperature to 38.17 ± 0.05 °C, 37.97 ± 0.05 °C and 37.91 ± 0.05 °C, respectively after 6 h. MEBM's antipyretic activity at doses of 200

mg/kg ($p < 0.05$) and 400 mg/kg ($p < 0.01$) was higher than that of control. However, the standard drug, paracetamol 150 mg/kg demonstrated the excellent antipyretic activity ($p < 0.001$) compared with that of control. After 8 h, body temperature in the MEBM-treated groups (200 and 400 mg/kg) remained essentially unchanged at 38.21 ± 0.07 and 37.99 ± 0.04 °C, respectively.

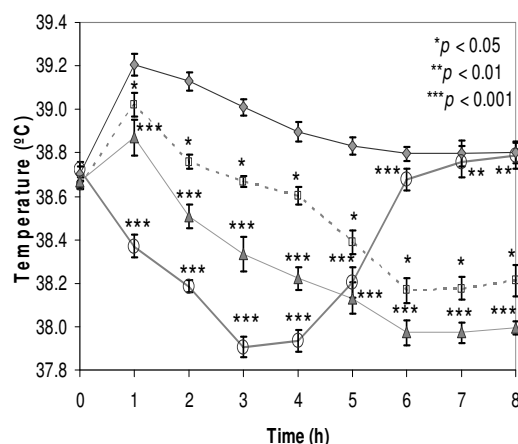


Figure 1: Antipyretic effect of MEBM on Baker's yeast-induced pyrexia in rats (♦ = control; □ = MEBM 200 mg/kg; ▲ = MEBM 400 mg/kg; ○ = paracetamol 150 mg/kg)

DISCUSSION

The present study showed that the methanol extract of *Bombax malabaricum* (MEBM) possessed significant antipyretic activity in Baker's yeast-induced pyrexia. The standard (paracetamol) achieved maximum antipyretic activity in 3 h; its activity decreased subsequently probably due to metabolism and excretion of the drug. On the other hand, maximum antipyretic activity for MEBM occurred at 6 h, indicating slow but steady absorption of the drug from the GIT; this may have been responsible for the prolonged action of the extract. Subsequently, up to the 8th hour, its activity remained largely unchanged. The antipyretic activity of the extract was dose-dependent with the higher dose producing greater activity.

The hypothalamus regulates body temperature with a delicate balance between heat production and heat loss through the set-point control. Infection, tissue damage, inflammation, graft rejection, malignancy and several other ill-health conditions may elevate the set point to induce fever [12]. When the set point is raised, enhancement of the body temperature takes place through active generation and retention of heat. Vasoconstriction also helps to reduce heat loss through skin. In this way, the body matches the brain blood temperature with the new set point made by the hypothalamus [1]. Biochemically, during fever, enhanced formation of cytokines such as interleukins (IL-1 α , IL-1 β , IL-6 and IL-6), interferon (α , β), tumour necrosis factor alpha (TNF- α) takes place under such physiological conditions [12]. These cytokine factors migrate to circumventricular organs of the brain and bind with endothelial receptors on vessel walls or interact with local microglial cells. After binding, it activates the arachidonic acid pathway which enhances the synthesis of prostaglandin E₂ (PGE₂). The pathway consists of the enzymes phospholipase A₂, cyclo-oxygenase-2 (COX-2) and prostaglandin E₂ synthase, which are responsible for the synthesis and release of PGE₂. PGE₂ is the final mediator for febrile response. The set point temperature of the body remains elevated until PGE₂ is present in the hypothalamus [1]. Again, PGE₂ triggers the hypothalamus for more formation of heat by minimizing heat loss through cyclic adenosine mono-phosphate (cAMP) pathways [12]. It has been established that yeast induces pathogenic fever in rat by enhancing the production of prostaglandins, mainly PGE₂, which elevates the set point of the thermoregulatory centre in hypothalamus [13].

Paracetamol possesses potent antipyretic and analgesic activities with minimal anti-inflammatory activity. It may selectively inhibit specific COX isoform in the CNS to inhibit prostaglandin synthesis to achieve its antipyretic effect [14] but does not influence

body temperature when it is elevated by factors such as exercise or increase in ambient temperature [12]. Certain phytochemical compounds such as steroids, carbohydrates, tannins, triterpenoids, flavonoid and coumarin glycosides were found to be present in the extract during phytochemical screening. The antipyretic potentials of steroids, tannins, triterpenoids, flavonoid and coumarin glycosides have been reported in various studies [15-18]. Therefore, the antipyretic activity of MEBM may be due to its contents of steroids, tannins, triterpenoids, flavonoid and coumarin glycosides.

Furthermore, indirect evidence seems to support the influence of MEBM on the biosynthesis of prostaglandin (PGE₂) which is a regulator of body temperature; this may also partly account for its antipyretic activity in yeast-induced pyrexia model [1,11].

CONCLUSION

The results obtained demonstrate the significant antipyretic activity of the methanol extract of *Bombax malabaricum* leaves. Inhibition of the synthesis and/or release of inflammatory mediators may be its main mechanism(s) of action. These results also suggest that the presence of certain bioactive molecules may partly be responsible for the reported antipyretic activity of MEBM, the isolation of which could help to obtain improved antipyretic drugs with specific mechanism of action. Further experimentation is under way in our laboratory to isolate the active molecules from MEBM and to establish the exact mechanism of action of the extract.

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